including detailed histologic, gene, and protein expression profiles of explanted valves, will allow additional insight into cellular and molecular mechanisms of the observed in vivo remodeling of engineered heart valves. Additionally, we plan rigorous evaluation of valves constructed identically, followed by bioreactor conditioning. Although important questions remain regarding the types of scaffold materials and seeded cell types optimally used for heart valve tissue engineering, the knowledge gained from the current experiments will guide the next generation of valve design criteria and future progress toward engineered valves for pediatric application.[20]

We acknowledge the intellectual and technical contributions of Hugo Loyola (statistics and figures), William Regan and the Children's Hospital Boston Perfusion Team (cardiopulmonary bypass), Ryan Conway, Kimberlie Hauser, Mark Kelly, Katie Mullen, and Hugh Simonds (animal care), David Annese (MRI technical support), and Joyce Bischoff (laboratory methods and experimental design), without whom this work would not have been possible.

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Discussion

Dr Sunjay Kaushal *(Chicago, Ill)*. I really enjoyed that talk. I know these are very difficult experiments to perform. Could you provide more details on the histology? For instance, was there actual elastin formation at 20 weeks or how was the collagen organized?

Dr Gottlieb. At this point we have hypotheses but no evidence, and we are involved in detailed investigation of these specific topics, including the presence or absence of elastin at all time points and a description of the evolution of collagen in explanted tissues, including collagen maturity and fiber orientation. Unfortunately, I do not have those data available today.

Dr Kaushal. Was there growth in these valves over time? I know it is a short period of time, but any hints that the valves were growing?

Dr Gottlieb. We still are limited by sample size. Although this is a large number of sheep for a preclinical study, we are still limited by the statistical power of small samples. Therefore, we saw no difference between the time of implant and the time of explant over 20 weeks. However, we started with conduits that were oversized for the native pulmonary artery, and by the time the 20-week implants occurred, the native artery diameter and the conduit diameter were matched. We did not observe a time point at which there would be wall stress on the tissue-engineered conduit, which might result in growth.

Dr Michael E. Jessen *(Dallas, Tex)*. How much growth was there in the animal? What kind of weight gain did it have over the 20 weeks?

Dr Gottlieb. We implanted at 20 kg and explanted at 40 to 45 kg.

Dr. Jessen. So they doubled in size over that time interval?

Dr Gottlieb. That's right.

Dr Jessen. Did you make any measurements of what happened to the actual scaffolding material, the polyglycolic acid or the poly-L-lactic acid?

Dr Gottlieb. That is an important point, particularly in light of the area of the leaflet decreasing over time. We wonder whether this represents loss of polymer, extracellular matrix, and/or cells, or whether it represents a contraction or shrinkage of the tissue and a stable number of fibers of the bioresorbable scaffolds that are compressed into a smaller area. These are questions that will undergo investigation in the short term.

Dr David Kalfa *(Marseille, France)*. I really congratulate you for this work, and I have 2 questions for you. First, concerning

the extracellular matrix Dr Kaushal just spoke about, did you evaluate the presence of elastic fibers in the extracellular matrix, at least on a qualitative way, while waiting for detailed investigation to be performed? Second, were you interested in cellular tracking?

Dr Gottlieb. Both are excellent questions. The elastin question I have already spoken to. We do not have the complete data set yet on elastin in all explanted tissues at all time points, so I am reluctant to give information. But our preliminary data did not show much elastin.

In terms of your second question about tracking cells, in our laboratory previously there were experiments with small-scale scaffolds that were engineered into patches in which cells were tracked. We have undertaken some other experiments on a mouse scale where we can track cells using GFP labels. However, in our sheep model, we require that the valved conduits produce sufficient extracellular matrix to be hemostatic when implanted into the circulation. It is established that introduction of labeling vectors can cause changes in expression of a lot of different genes, not just in the addition of a label. After transfecting our bone marrow–derived MSCs with a GFP label, they made insufficient matrix to be hemostatic in the circulation. Because of this limitation, labeled valved conduits were unable to withstand pulmonary pressure. As a result, we have not undertaken those projects because we have not found a way yet to durably and accurately label cells in a large animal analogous model.

Dr John S. Ikonomidis *(Charleston, SC).* These results are clearly very encouraging because they illustrate the application of some difficult technology, but at the same time they are disappointing because of the observation of failure of these conduits after only 20 weeks. My first question is, have you followed these animals out longer to track the progression of conduit failure?

Second, can you speculate whether cations will be required to prolong conduit durability?

Dr Gottlieb. Those are tough questions. To the first one, our longest duration in our laboratory to date has been 20 weeks, which is 5 months. We see sufficient pulmonary regurgitation at that point to end experiments. We see right ventricular dilation, so evidence of relatively long-term valve regurgitation. We have not monitored them longer than that. We are hopeful that we will have valve leaflets that function better in the future, and we will then undertake those longer-term experiments.

In terms of the disappointment associated with failure of the valves, I think we can only make progress through an iterative process in which we look at the modes of failure in an engineering approach. Our first analysis now is to try to understand what went wrong. Our second move would be to identify the biology resulting in valve failure, then to manipulate it in vitro, and then to try it again in vivo.

Dr Jolanda Kluin *(Utrecht, The Netherlands).* Could you briefly compare your results with the results of the 2000 paper by Simon Hoerstrup of your institution?

Dr Gottlieb. Dr Hoerstrup's work involved a polymer of polyglycolic acid that was dip coated in poly-4-hydroxybutyrate and seeded with vascular cells. They were completely differentiated cells from the carotid artery smooth muscle cells and endothelial cells. To start, we had completely different models, both in terms of scaffold polymer and in terms of cell. These variables are nontrivial and can account for many differences that we have seen in our preliminary analysis of these valves. His experiments involved 6 animals, 1 at each of 6 different time points. Our current experiment is different because it involves a greater number of animals at multiple time points. His was the proof-of-concept experiment; our current work represents the test of statistical reproducibility. In the current experiments, we have seen evidence of valve regurgitation, which was potentially similar to the central valvular regurgitation, mentioned but not quantitated in the Hoerstrup paper. This builds on that work or the model that was established in that work with different cell types and different scaffolds.

In terms of histology, we will have an opportunity to do a very in-depth analysis and to report those results separately.